Mobile phase: MeOH:water 90:10 + 1% formic acid, pH 3.5
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Sauer,B.; Matusch,R. High-performance liquid chromatographic separations of nystatin and their influence on the antifungal activity, *J.Chromatogr.A*, **1994**, *672*, 247–253.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ODS-Hypersil
Mobile phase: MeOH:DMF:10 mM pH 7.0 Tris in water 56:9.6:34.4
Detector: UV 305

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Egodage,K.L.; Haslam,J.S.; Rajewski,R.A.; Stella,V.J. Correlation and validation to the USP bioassay of a RP-HPLC assay for nystatin (Abstract 3373), *Pharm.Res.*, **1997**, *14*, S587.

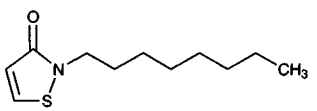
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in DMSO to 10 mg/mL, dilute 1:20 with MeOH.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:50 mM phosphate buffer (pH 3.5-8.1) 30:70 to 35:65
Flow rate: 0.4-2
Detector: UV 313

OTHER SUBSTANCES
Simultaneous: amphotericin A

REFERENCE
Aszalos,A.; Bax,A.; Burlinson,N.; Roller,P.; McNeal,C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A, *J.Antibiot.(Tokyo)*, **1985**, *38*, 1699–1713.

Octhilinone



Molecular formula: $C_{11}H_{19}NOS$
Molecular weight: 213.34
CAS Registry No.: 26530-20-1
Merck Index: 6853

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

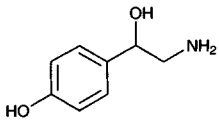
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.112

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Octopamine



Molecular formula: $C_8H_{11}NO_2$
Molecular weight: 153.18
CAS Registry No.: 104-14-3, 876-04-0 (D-(-)), 770-05-8 (DL HCl)
Merck Index: 6856
Lednicer No.: 5 23

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: 0.1% pH 1.9 Perchloric acid
Column temperature: 25
Flow rate: 1
Detector: UV 210

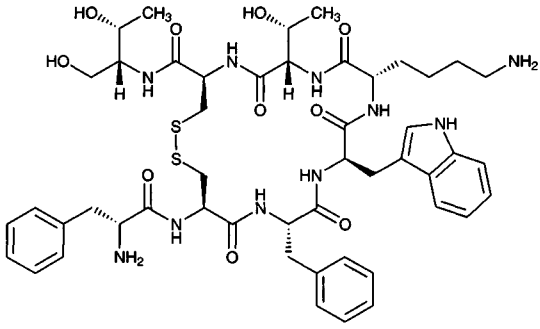
CHROMATOGRAM
Retention time: 4.64, 4.95

OTHER SUBSTANCES
Simultaneous: norepinephrine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Octreotide



Molecular formula: $C_{49}H_{66}N_{10}O_{10}S_2$
Molecular weight: 1019.26
CAS Registry No.: 83150-76-9
Merck Index: 6859

SAMPLE
Matrix: bile, blood, feces, urine
Sample preparation: Hydrolyze pooled plasma with 5 mg Subtilisin A at 50° for 1 h. Lyophilize feces and extract twice with 4 volumes MeOH. Lyophilize urine and bile and reconstitute with water.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 (Brownlee)
Column: 100 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: Gradient. MeCN:water:trifluoroacetic acid from 0:99.8:0.2 to 99.8:0:0.2 over 40 min.
Flow rate: 1.5
Detector: UV 210 or radioactivity

CHROMATOGRAM
Retention time: 45

KEY WORDS
rat; plasma

REFERENCE
Lemaire,M.; Azria,M.; Dannecker,R.; Marbach,P.; Schweitzer,A.; Maurer,G. Disposition of sandostatin, a new synthetic somatostatin analogue, in rats, *Drug Metab.Dispos.*, **1989**, *17*, 699–703.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.6 5 μm Bakerbond C18
Mobile phase: Gradient. A was MeCN:water:1 M tetramethylammonium hydroxide 10:88:2 adjusted to pH 4.5 with concentrated orthophosphoric acid. B was MeCN:water:1 M tetramethylammonium hydroxide 60:38:2 adjusted to pH 4.5 with concentrated orthophosphoric acid. A:B from 100:0 to 0:100 over 14 min.
Flow rate: 1.3
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: degradation products, des-threninol

KEY WORDS
protect from light; injections

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Resztak,K.E.; Prince,S.J. Stability of octreotide acetate in polypropylene syringes, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2356–2358.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb RP18
Mobile phase: MeCN:buffer 31:69, pH 4.7 (Buffer was 20 mM tetramethylammonium hydroxide adjusted to pH 4.7 with phosphoric acid.)
Column temperature: 40
Flow rate: 1.2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: derivatives

REFERENCE
Kuhn,R.; Morin,C.; Erni,F. A simple model describing the retention behavior of octreotide and its glycosylated derivatives in reversed phase HPLC, *Chromatographia*, **1995**, *41*, 516–520.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spheri-5 C18
Mobile phase: MeCN:water:1 M tetramethylammonium hydroxide pentahydrate 33:65:2, adjusted to pH 4.5 with phosphoric acid
Flow rate: 0.8
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 6.2

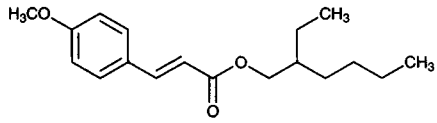
OTHER SUBSTANCES
Simultaneous: degradation products (UV 210)

KEY WORDS
injections

REFERENCE
Ripley,R.G.; Ritchie,D.J.; Holstad,S.G. Stability of octreotide acetate in polypropylene syringes at 5 and -20°C, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1910–1911.

Octyl methoxycinnamate

Molecular formula: $C_{18}H_{26}O_3$
Molecular weight: 290.40
CAS Registry No.: 5466-77-3
Merck Index: 6864



SAMPLE

Matrix: formulations
Sample preparation: 1-1.5 g sun-screen lotion + 50 mL isopropanol, dissolve. Remove a 5 mL aliquot and make up to 50 mL with mobile phase, filter (0.45 μm) inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm C8 Hypersil
Mobile phase: Isopropanol:buffer 10:90 (Buffer was 100 mM sodium dodecyl sulfate (electrophoresis grade) containing 0.3% triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254, UV 300

CHROMATOGRAM

Retention time: 19.40

OTHER SUBSTANCES

Simultaneous: 2-ethylhexyl p-dimethylaminobenzoate, oxybenzone, methyl paraben, propyl paraben

KEY WORDS

lotion

REFERENCE

Tomasella,F.P.; Zuting,P.; Love,L.J. Determination of sun-screen agents in cosmetic products by micellar liquid chromatography, *J.Chromatogr.*, **1991**, *587*, 325–328.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out lotion, add 20 mL EtOH, heat to 60°, stir for 30 min at room temperature, make up to 25 mL, stir for 5 min, centrifuge at 14500 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 200 × 4.6 5 μm Ultrasphere C8
Mobile phase: Gradient. MeOH:1% aqueous acetic acid from 80:20 to 100:0 over 10 min, maintain at 100:0 for 2 min, re-equilibrate for 4 min.
Column temperature: 25
Flow rate: 1
Detector: UV 325

CHROMATOGRAM

Retention time: 8.7

OTHER SUBSTANCES

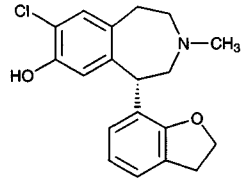
Simultaneous: benzophenone-3, butylmethoxydibenzoylmethane, impurities

KEY WORDS

lotion; sunscreen; an isomer forms on exposure to light

REFERENCE
Meijer,J.; Lodén,M. Stability analysis of three UV-filters using HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 1821–1832.

Odapipam



Molecular formula: $C_{19}H_{20}ClNO_2$
Molecular weight: 329.83
CAS Registry No.: 131796-63-9

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 500 mg C18 Bond-Elut SPE cartridge with MeOH and water. Mix 2 mL microsomal incubation with 4.5 mL ice-cold MeOH, centrifuge at 4000 g for 10 min, dilute the supernatant with 15 mL water. Add a 2 mL aliquot to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH:25% aqueous ammonia 96:4, evaporate the eluate to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm ChromSpher Si (Chrompack)
Mobile phase: n-Heptane:2-propanol:water:25% ammonia 90:10:0.2:0.1
Detector: UV 280; MS, VG TRIO 1000, particle beam interface at 50°, helium at 25-30 psi, ion source 200°, positive ionization mode, electron current 150 μA, electron energy 70 eV

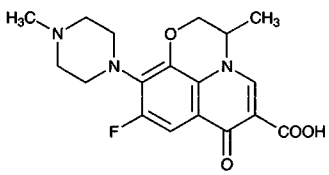
CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
normal phase; rat; liver; SPE

REFERENCE
Andersen,J.V.; Hansen,K.T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline, *Xenobiotica*, **1997**, *27*, 901–912.

Ofloxacin



Molecular formula: $C_{18}H_{20}FN_3O_4$
Molecular weight: 361.37
CAS Registry No.: 82419-36-1
Merck Index: 6865
Lednicer No.: 4 141-145

SAMPLE
Matrix: aqueous humor, blood
Sample preparation: Aqueous humor. Inject a 10 μL aliquot directly. Plasma. Condition a 3 mL C18 SPE cartridge (Varian) with two 3 mL portions of MeCN and 3 mL buffer. Add 2 mL 625 ng/mL ciprofloxacin in buffer to 500 μL of plasma, mix, add to the SPE cartridge. Wash with 3 mL buffer. Remove moisture with vacuum (200 mbar) for 10 min. Elute with two 500 μL

portions of MeCN:buffer 40:60. Vortex the eluate, inject a 10 μL aliquot. (Buffer was 100 mM Tris adjusted to pH 5.0 with HCl).

HPLC VARIABLES
Column: 300 × 4.6 5 μm endcapped ODS-Hypersil
Mobile phase: MeCN:DMF:10 mM NaH_2PO_4 15:6:79, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 285

CHROMATOGRAM
Retention time: 10.2
Internal standard: ciprofloxacin (12.0)
Limit of detection: 80 ng/mL (aqueous humor), 310 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: cefotaxime

KEY WORDS
plasma; SPE

REFERENCE
Kraemer,H.-J.; Gehrke,R.; Breithaupt,A.; Breithaupt,H. Simultaneous quantification of cefotaxime, desacetylcefotaxime, ofloxacine and ciprofloxacine in ocular aqueous humor and in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 147–153.

SAMPLE
Matrix: bile, blood, perfusate
Sample preparation: Intestinal perfusate. Centrifuge before analysis. Plasma. Mix 200 μL plasma with 200 μL 100 mM pH 6.8 phosphate buffer, add 4 mL dichloromethane, shake at 100 cycles/min for 10 min. Remove the organic layer and dry it under nitrogen at 40°, reconstitute with 200 μL mobile phase, inject a 20 μL aliquot (J. Chromatogr. 1988, 434, 320). Bile. Mix 100 μL bile with 900 μL pH 7.0 phosphate buffer, add 500 μL MeOH. Shake for 1 min and centrifuge at 1000 g for 5 min. Inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: Bovine Serum Albumin (Macherey Nagel)
Mobile phase: 200 mM Potassium dihydrogen phosphate containing 5 mM N,N-dimethyloctylamine, adjusted to pH 8.0 with KOH
Flow rate: 1
Injection volume: 20
Detector: F ex 298 em458

CHROMATOGRAM
Retention time: 6 (S-(-)), 7.8 (R-(+))
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Also analyzed: cefoperazone, ciprofloxacin, ofloxacin, quinidine, verapamil

KEY WORDS
chiral; intestinal efflux; pharmacokinetics; plasma

REFERENCE
Rabbaa,L.; Dautrey,S.; Colas-Linhart,N.; Carbon,C.; Farinotti,R. Intestinal elimination of ofloxacin enantiomers in the rat: Evidence of a carrier-mediated process, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2126–2130.

SAMPLE
Matrix: blood

Sample preparation: Mix 1 mL blood with 1 mL 0.25 mM Triton, vortex for 30 s, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutyl ammonium hydrogen sulfate, adjusted to pH 3.0 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 5
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; pharmacokinetics; rabbit

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: Mix equal volumes serum and MeOH. Centrifuge sample, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 Spherisorb 5 ODS II
Mobile phase: MeCN:water:phosphoric acid 5:94.6:0.32 adjusted to pH 3.0 with tetrabutylammonium hydroxide
Column temperature: 50
Flow rate: 2
Injection volume: 20
Detector: F ex 310 em 489

CHROMATOGRAM
Limit of detection: 80 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Bethell,D.B.; Day,N.P.; Dung,N.M.; McMullin,C.; Loan,H.T.; Tam,D.T.; Minh,L.T.; Linh,N.T.; Dung,N.Q.; Vinh,H.; MacGowan,A.P.; White,L.O.; White,N.J. Pharmacokinetics of oral and intravenous ofloxacin in children with multidrug-resistant typhoid fever, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2167–2172.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood or 500 μL plasma, vortex for 30 s with 1 mL 250 μM Triton, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min. Inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was mixture of 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutylamonium hydrogen sulfate, adjusted to pH 3 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 5
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Blood or 500 μL plasma, vortex for 30 s with 1 mL 250 μM Triton, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min. Inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was mixture of 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutylamonium hydrogen sulfate, adjusted to pH 3 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 277 em 445

CHROMATOGRAM
Retention time: 9
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 295

CHROMATOGRAM
Retention time: 8.54
Internal standard: pipemic acid (4.67)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 295

CHROMATOGRAM

Retention time: 8.648

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Dilute a 25 mg/mL levofloxacin injection with an infusion fluid to a concentration of 100 μg/mL. Inject a 20 μL aliquot. (Infusion fluids were 0.9% NaCl, 5% dextrose, 5% dextrose and 0.9% NaCl, 5% dextrose and lactated Ringer's injections, 5% sodium bicarbonate, Plasma-Lyte 56 and 5% dextrose, 5% dextrose, 0.45% NaCl, and 0.15% KCl, 1/6 M sodium lactate, sterile water, and 20% mannitol.)

HPLC VARIABLES

Column: 250 × 4.6 Zorbax SB-Phenyl

Mobile phase: MeCN:MeOH:94 mM KH_2PO_4:trifluoroacetic acid 15:5:80:0.3

Column temperature: 45

Flow rate: 1

Injection volume: 25

Detector: UV 294

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

injections; stability indicating; for levofloxacin

REFERENCE

Williams,N.A.; Bornstein,M.; Johnson,K. Stability of levofloxacin in intravenous solutions in polyvinyl chloride bags, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2309–2313.

SAMPLE
Matrix: growth medium
Sample preparation: 500 μL Sample + 500 μL 100 μg/mL IS in cold (4°) MeCN, vortex, centrifuge at 3000 *g* for 5 min. Remove a 500 μL aliquot of the supernatant, filter (0.45 μm Acrodisc syringe filter), inject a 30 μL aliquot. (Protect all specimens from light.)

HPLC VARIABLES
Guard column: C18 5U (Alltech)
Column: 150 × 4.6 7 μm Adsorbosphere HS C18 7U
Mobile phase: MeCN:20 mM pH 3.0 phosphate buffer 35:65 containing 0.2% triethylamine and 0.2% sodium dodecyl sulfate, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1.75
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 4.15
Internal standard: sparfloxacin (7.09)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Also analyzed: ciprofloxacin, clinafloxacin, levofloxacin, sparfloxacin, temafloxacin, trovafloxacin

KEY WORDS
Mueller-Hinton broth

REFERENCE
Wright,D.H.; Herman,V.K.; Konstantinides,F.N.; Rotschafer,J.C. Determination of quinolone antibiotics in growth media by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *709*, 97–104.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 4 μm NovaPak C18
Mobile phase: MeCN:MeOH:buffer:acetic acid 2.5:10:86.5:1 containing 20 mM triethylamine (The pH 2.7 buffer was 0.4% diammonium hydrogen phosphate in water containing 0.4% (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Detector: UV 279

CHROMATOGRAM
Retention time: 11.6

OTHER SUBSTANCES
Extracted: ciprofloxacin, enrofloxacin

REFERENCE
Cester,C.C.; Toutain,P.L. A comprehensive model for enrofloxacin to ciprofloxacin transformation and disposition in dog, *J.Pharm.Sci.*, **1997**, *86*, 1148–1155.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 295

CHROMATOGRAM
Retention time: 8.8

OTHER SUBSTANCES
Simultaneous: ciprofloxacin (UV 280), enoxacin (UV 280), fleroxacin (UV 280), norfloxacin (UV 280), pipemidic acid (UV 280)

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography. Prediction of retention of a series of quinolones, *J.Chromatogr.A*, **1996**, *719*, 27–36.

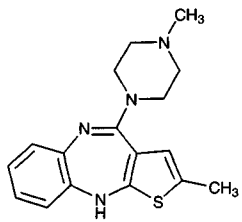
SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S (Supelco)
Column: 250 × 4.6 Suplecosil LC-18S
Mobile phase: MeCN:buffer:water 10:3.5:86.5 (Buffer was 400 mM tetrabutylammonium hydroxide adjusted to pH 2.85.)
Flow rate: 1.8
Detector: UV 280

REFERENCE
Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

Olanzapine

Molecular formula: $C_{17}H_{20}N_4S$
Molecular weight: 312.44
CAS Registry No.: 132539-06-1
Merck Index: 6959



SAMPLE
Matrix: blood
Sample preparation: Condition a Certify mixed bed (RP and ion exchange) SPE cartridge with MeOH and phosphate buffer. Add serum, wash with 1 M acetic acid, wash with MeOH, elute with 3% ammonium hydroxide in ethyl acetate. Evaporate the eluate, reconstitute, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Microsorb CN
Mobile phase: MeCN:MeOH:50 mM pH 6.5 sodium phosphate buffer 5:28:67
Column temperature: 37
Flow rate: 1.5

CHROMATOGRAM
Retention time: 9.6
Internal standard: clozapine (12.5)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: imipramine
Also analyzed: haloperidol, risperidone
Interfering: paroxetine

KEY WORDS
SPE; serum

REFERENCE
Prieto,I.V.; Hoffman,D.W. HPLC monitoring of olanzapine (Abstract 131), *Ther.Drug Monit.*, **1997**, *19*, 580.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL Plasma with 100 μL 100 ng/mL IS in MeCN, add 500 μL saturated sodium carbonate, mix. Add 7 mL pentane:dichloromethane 85:15, shake for 10 min, centrifuge at 18° for 10 min. Evaporate the supernatant to dryness under a slow stream of nitrogen at 60°. Dissolve the residue in 150 μL MeCN, inject a 30-120 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere CN
Mobile phase: MeCN:MeOH:130 mM pH 6.8 ammonium acetate 86:6:8
Column temperature: 40
Flow rate: 0.8
Injection volume: 30-120
Detector: E, ESA Coulochem model 5100A, model 5020 guard cell +1 V, model 5011 dual electrode analytical cell, electrode 1 +300 mV, electrode 2 +930 mV

CHROMATOGRAM
Retention time: 14.6
Internal standard: 2-ethyl analog of olanzapine LY170222 (Lilly Research Labs) (13)
Limit of detection: 250 pg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, benztropine, chlorpromazine, clonazepam, clozapine, fluphenazine, ibuprofen, lorazepam, perphenazine, pseudoephedrine, risperidone, spiperone, sulpiride, trifluoperazine, trihexyphenidyl

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Aravagiri,M.; Ames,D.; Wirshing,W.C.; Marder,S.R. Plasma level monitoring of olanzapine in patients with schizophrenia: Determination by high-performance liquid chromatography with electrochemical detection, *Ther.Drug Monit.*, **1997**, *19*, 307–313.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 200 μL cold MeCN, mix, centrifuge in a microfuge at maximum speed for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm YMC basic column (YMC, USA)
Mobile phase: MeCN:MeOH:75 mM pH 7 sodium phosphate buffer 20:30:50
Column temperature: 40
Flow rate: 1
Detector: E, ESA Coulochem Dual Electrode Detector, guard cell model 5020 + 300 mV, analytical cell model 5014, detector 1 + 0.0 V, detector 2 + 300 mV

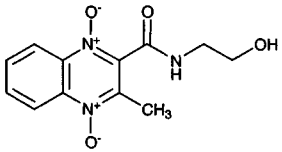
CHROMATOGRAM
Retention time: 12
Internal standard: LY170222 (2-ethylolanzapine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; human

REFERENCE
Ring,B.J.; Catlow,J.; Lindsay,T.J.; Gillespie,T.; Roskos,L.K.; Cerimele,B.J.; Swanson,S.P.; Hamman,M.A.; Wrighton,S.A. Identification of the human cytochromes P450 responsible for the in vitro formation of the major oxidative metabolites of the antipsychotic agent olanzapine, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 658–666.

Olaquindox



Molecular formula: $C_{12}H_{13}N_3O_4$
Molecular weight: 263.25
CAS Registry No.: 23696-28-8
Merck Index: 6960

SAMPLE
Matrix: feed
Sample preparation: Grind and sieve feed with a Moulinex blender. Weigh out 10 g and add it to 20 mL DMF and 60 mL carbon tetrachloride, stir magnetically at 500 rpm at 60° for 30 min, cool, filter (100 μm glass), wash the residue with a little carbon tetrachloride. Remove 25 mL of the filtrate and add it to 45 mL water, stir vigorously for 2 min, centrifuge at 320 g for 5 min, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: Gradient. MeOH:water 15:85 for 4 min, to 50:50 over 2 min, maintain at 50:50 for 4 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 262

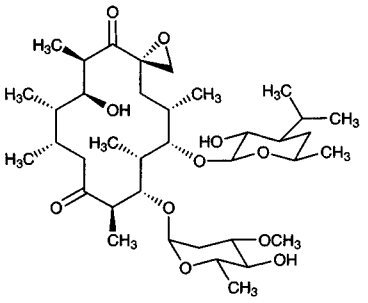
CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: carbadox (UV 305 nm)

KEY WORDS
protect from light

REFERENCE
dos Ramos,F.J.; da Silveira,I.N.; de Graaf,G. Column liquid chromatographic determination of carbadox and olaquindox in feeds, *J.Chromatogr.*, **1991**, *558*, 125–130.

Oleandomycin



Molecular formula: $C_{35}H_{61}NO_{12}$
Molecular weight: 687.87
CAS Registry No.: 3922-90-5, 6696-47-5 (HCl), 7060-74-4 (phosphate)
Merck Index: 6962

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize (Physcotron) liver with 4 volumes of ice-cold saline. Put 200 μL plasma or liver homogenate into the tube. Add 2 mL MTBE and 5 μL 1 M NaOH and shake mechanically for 5 min. Centrifuge at 1500 g for 10 min, transfer the upper layer into a glass tube and evaporate it to dryness under dry nitrogen. Rinse the inner wall of the tube with 200 μL MeOH and evaporate to dryness. Dissolve the residue in 30 μL MeOH and inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5-C18 (Nacalai Tesque)
Mobile phase: MeCN:100 mM pH 6.6 sodium acetate buffer 50:50
Flow rate: 0.6
Injection volume: 10
Detector: E, BAS LC-4C, 1.1 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Extracted: erythromycin

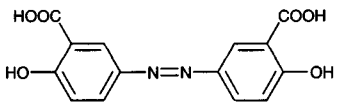
KEY WORDS

rat; plasma; liver; pharmacokinetics; oleandomycin is IS

REFERENCE

Hanada,E.; Ohtani,H.; Kotaki,H.; Sawada,Y.; Iga,T. Determination of erythromycin concentrations in rat plasma and liver by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1997**, *692*, 478–482.

Olsalazine



Molecular formula: $C_{14}H_{10}N_2O_6$
Molecular weight: 302.24
CAS Registry No.: 15722-48-2, 6054-98-4 (Na salt)
Merck Index: 6976
Lednicer No.: 4 42

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Add disodium-3,5'-azo-bis-(6-hydroxybenzoate) to serum, treat with proteinase K (0.5 mg/mL protein) for 10 min, add tetrabutylammonium hydrogen sulfate buffered to pH 6.5, add dichloromethane, agitate for 30 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

Urine. Add 2,4-dihydroxybenzoic acid to urine, add perchloric acid, add diethyl ether, shake for 10 min, freeze. Remove the organic layer and add it to pH 7.4 phosphate buffer, extract, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP-18
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:buffer 52:48 (Buffer was pH 7.4 phosphate buffer containing 20 mM tetrabutylammonium hydrogen sulfate.)
Detector: UV 365

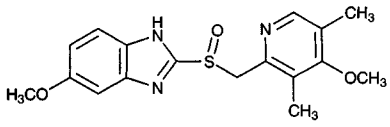
CHROMATOGRAM
Retention time: k' 5.2
Internal standard: disodium-3,5'-azo-bis-(6-hydroxybenzoate), 2,4-dihydroxybenzoic acid
Limit of quantitation: 1 μM urine, 0.5 μM (serum)

OTHER SUBSTANCES
Extracted: olsalazine sulfate

KEY WORDS
serum; pharmacokinetics

REFERENCE
Ryde,E.M.; Ahnfelt,N.-O. The pharmacokinetics of olsalazine sodium in healthy volunteers after a single i.v. dose and after oral doses with and without food, *Eur.J.Clin.Pharmacol.*, **1988**, *34*, 481–488.

Omeprazole



Molecular formula: $C_{17}H_{19}N_3O_3S$
Molecular weight: 345.42
CAS Registry No.: 73590-58-6
Merck Index: 6977
Lednicer No.: 4 133

SAMPLE
Matrix: blood
Sample preparation: Extract 500 μL plasma with dichloromethane containing 50 μL 1.0 M dibasic sodium phosphate and 100 μL 25 μg/mL IS. Centrifuge and aspirate aqueous layer to waste. Evaporate organic layer under a stream of nitrogen, reconstitute residue in 200 μL MeCN:20 mM pH 8.0 phosphate buffer 25:75. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Zorbax SB C18
Mobile phase: Gradient. MeCN:20 mM pH 7.5 phosphate buffer from 30:70 to 40:60 over 10 min. (Mobile phase was contained 0.1% triethylamine.)
Injection volume: 10
Detector: UV 302, to UV 280 at 6.4 min

CHROMATOGRAM
Retention time: 5.7
Internal standard: carbamazepine (7.3)

KEY WORDS
plasma

REFERENCE
Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 72 μg/mL flunitrazepam in MeOH to 1 mL plasma, shake briefly, add 3 mL toluene:isoamyl alcohol 95:5, vortex at 1000 rpm for 90 s, centrifuge at 2600 g for 10 min. Evaporate a 2.5 mL aliquot of the upper organic layer to dryness under nitrogen at 40°, reconstitute with 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Nucleosil 120-5 C18
Column: 250 × 4 5 μm Nucleosil 120-5 C 18
Mobile phase: MeOH:buffer 47:53 (Buffer was 100 mM Na_2HPO_4 adjusted to pH 7.8 with orthophosphoric acid.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 25
Detector: UV 302

CHROMATOGRAM
Retention time: 10.1
Internal standard: flunitrazepam (11.4)
Limit of quantitation: 9.7 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Macek,J.; Ptácek,P.; Klíma,J. Determination of omeprazole in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *689*, 239–243.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

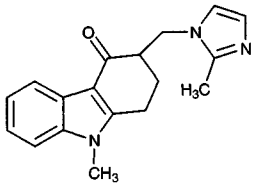
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.065

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ondansetron



Molecular formula: $C_{18}H_{19}N_3O$
Molecular weight: 293.37
CAS Registry No.: 99614-02-5, 116002-70-1, 99614-01-4 (HCl dihydrate), 103639-04-9 (HCl dihydrate)
Merck Index: 6979
Lednicer No.: 5 164

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:20 mM KH_2PO_4 43:57
Flow rate: 1.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.6

OTHER SUBSTANCES
Simultaneous: idarubicin

KEY WORDS
0.9% NaCl; injections

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determination of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 979–988.

SAMPLE
Matrix: formulations
Sample preparation: If necessary, dilute injection 1:9 with mobile phase (for 50 mL admixtures) and 1:4 (for 100 mL admixtures), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select B
Mobile phase: MeCN:buffer 25:75 (Buffer was 20 mM KH_2PO_4 adjusted to pH 6.0 with NaOH solution.)
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: dexamethasone

KEY WORDS
5% dextrose; 0.9% sodium chloride; injections; stability-indicating

REFERENCE
Evrard,B.; Ceccato,A.; Gaspard,O.; Delattre,L.; Delporte,J.-P. Stability of ondansetron hydrochloride and dexamethasone sodium phosphate in 0.9% sodium chloride injection and in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 1065–1068.

SAMPLE
Matrix: solutions
Sample preparation: Add 380 μg propofol and 83 μg ondansetron hydrochloride to 0.9% sodium chloride, shake vigorously for 2 min, make up to 10 mL with 0.9% sodium chloride, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm T-Bondapak phenyl
Mobile phase: MeCN:buffer 50:50 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 8.5
Limit of detection: 61 ng/mL

OTHER SUBSTANCES
Simultaneous: propofol

REFERENCE
King,D.T.; Stewart,J.T.; Venkateshwaran,T.G. HPLC determination of propofol-thiopental sodium and propofol-ondansetron mixtures, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2285–2294.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in 0.9% sodium chloride.

HPLC VARIABLES
Column: 220 × 4.6 5 μm underivatized silica (Brownlee Silica Applied Biosystems, Inc.,San Jose)
Mobile phase: MeOH:buffer 40:60 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.7
Limit of detection: 47 ng/mL

OTHER SUBSTANCES
Simultaneous: meperidine, morphine (UV 233)

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T.; King,D.T. HPLC determination of morphine-ondansetron and meperidine-ondansetron mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1329–1338.

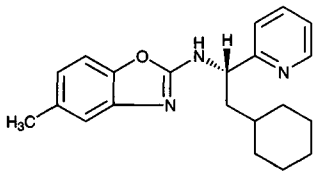
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 40:60
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

KEY WORDS
14C labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

Ontazolast



Molecular formula: $C_{21}H_{25}N_3O$
Molecular weight: 335.45
CAS Registry No.: 147432-77-7

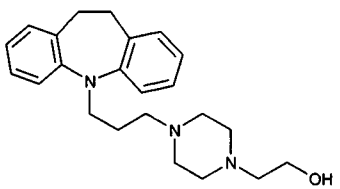
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax LC-8
Mobile phase: MeCN:MeOH:50 mM pH 6 phosphate buffer 1:3:8
Flow rate: 2
Detector: UV 245

CHROMATOGRAM
Retention time: 7
Internal standard: BIRM 390 BS (10.5)
Limit of quantitation: 5 ng/mL

REFERENCE
Hauss,D.J.; Fogal,S.E.; Ficorilli,J.V.; Price,C.A.; Roy,T.; Jayaraj,A.A.; Keirns,J.J. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor, *J.Pharm.Sci.*, **1998**, *87*, 164–169.

Opipramol



Molecular formula: $C_{23}H_{29}N_3O$
Molecular weight: 363.50
CAS Registry No.: 315-72-0, 909-39-7 (2.HCl)
Merck Index: 6985

SAMPLE
Matrix: blood
Sample preparation: Deproteinize 100 μL plasma with 100 μL MeCN containing 2.0 mg/L IS. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 Superspher

Column: 125 × 4 Superspher 60 select B
Mobile phase: MeCN:0.07% orthophosphoric acid 20:80
Column temperature: 30
Flow rate: 1.2
Injection volume: 50
Detector: UV 210, UV 255

CHROMATOGRAM
Retention time: 4.1
Internal standard: methylphenyl-phenylhydantion (7.8)
Limit of detection: 50 μg/L

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Lappenberg-Pelzer,M. Identification and determination of opipramol metabolites in plasma and urine, *J.Anal.Toxicol.*, **1998**, *22*, 215–219.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 256

CHROMATOGRAM
Retention time: 7.62
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 255.8

CHROMATOGRAM

Retention time: 14.163

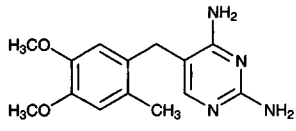
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ormetoprim



Molecular formula: $C_{14}H_{18}N_4O_2$
Molecular weight: 274.32
CAS Registry No.: 6981-18-6
Lednicer No.: 2 302

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 200 µL Plasma + 90 µL 24% trichloroacetic acid in MeOH + 10 µL 10 µg/mL sulfamethoxazole in MeOH, vortex for 30 s, centrifuge at 14000 g for 5 min, inject a 50 µL aliquot of the supernatant. Muscle. Homogenize 1 g muscle in 1.5 mL MeOH:buffer 20:80, add 50 µL 10 µg/mL sulfamethoxazole in MeOH, mix thoroughly for 1 min, centrifuge at 14000 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)

HPLC VARIABLES

Guard column: 20 × 4.6 40 µm ODS-Hypersil
Column: 150 × 4.6 3 µm ODS-Hypersil C18
Mobile phase: MeCN:buffer:triethylamine 20:80:0.02 (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 270

CHROMATOGRAM

Retention time: 12.5
Internal standard: sulfamethoxazole (8)
Limit of detection: 50 ng/g (muscle), 30 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: sulfadimethoxine

KEY WORDS

plasma; muscle; fish; salmon

REFERENCE

Samuelsen,O.B. Simultaneous determination of ormethoprim and sulphadimethoxine in plasma and muscle of Atlantic salmon *(Salmo salar)*, *J.Chromatogr.B*, **1994**, *660*, 412–417.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeCN:MeOH:100 mM pH 4.5 acetate buffer 12.5:12.5:75, inject an aliquot.

HPLC VARIABLES

Column: C18 (Rainin)
Mobile phase: MeCN:MeOH:50 mM phosphate buffer 12.5:12.5:75, pH 3.0
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: sulfamethoxazole, trimethoprim

REFERENCE
Brown,M.P.; Gronwall,R.; Castro,L. Pharmacokinetics and body fluid and endometrial concentrations of trimethoprim-sulfamethoxazole in mares, *Am.J.Vet.Res.*, **1988**, *49*, 918–922.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 5 g Fish + 1 mL 100 μg/mL carbamazepine diol in MeOH + 15 mL MeCN + 500 μL 50% trichloroacetic acid, homogenize (Brinkmann Polytron PT 10/35) at medium speed for 30 s, centrifuge at 4° at 7800 g for 25 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 5 mL water, vortex for 30 s, filter (13 mm dia. 8 μm Membra-Fil (Nucleopore)), add the filtrate to the SPE cartridge, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 20 μL aliquot. (Flush injection valve with 1 mL mobile phase between analyses.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18
Column: 250 × 4.6 5 μm Ultrasphere
Mobile phase: MeCN:MeOH:100 mM pH 4.0 phosphate buffer 17:10:73
Flow rate: 1
Injection volume: 20
Detector: UV 280

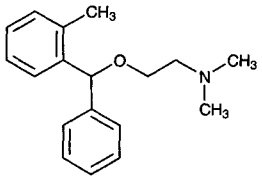
CHROMATOGRAM
Retention time: 5.5
Internal standard: carbamazepine diol (10.5)
Limit of quantitation: 0.2 ppm

OTHER SUBSTANCES
Extracted: sulfadimethoxine
Simultaneous: sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfisoxazole

KEY WORDS
fish; salmon; SPE; pharmacokinetics

REFERENCE
Walisser,J.A.; Burt,H.M.; Valg,T.A.; Kitts,D.D.; McErlane,K.M. High-performance liquid chromatographic analysis of Romet-30 in salmon following administration of medicated feed, *J.Chromatogr.*, **1990**, *518*, 179–188.

Orphenadrine



Molecular formula: $C_{18}H_{23}NO$
Molecular weight: 269.39
CAS Registry No.: 83-98-7, 4682-36-4 (citrate)
Merck Index: 7007
Lednicer No.: 1 42

SAMPLE
Matrix: blood
Sample preparation: Add 300 μL MeCN to 100 μL plasma, vortex, centrifuge for 2 min. Remove the supernatant and add it to 300 μL pH 5.9 sodium phosphate buffer and 3 mL hexane and vortex for 45 s. Centrifuge at 2500 g for 3 min. Evaporate the supernatant under a stream of nitrogen and reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 ODS

Mobile phase: MeCN:MeOH:25 mM potassium phosphate 25:25:50 containing 0.75 mL/L 2 M sulfuric acid and 0.25 mL/L triethylamine
Flow rate: 1.0
Detector: UV 250

CHROMATOGRAM
Retention time: 5.7
Internal standard: orphenadrine

OTHER SUBSTANCES
Extracted: ethopropazine

KEY WORDS
plasma; rat; pharmacokinetics; orphenadrine is IS

REFERENCE
Padovani,P.K.; Timby,D.M.; Wright,M.R.; Kapil,R.P. Quantitative analysis of DMP 851 in rat and dog plasma by liquid-liquid extraction and reverse-phase high performance liquid chromatography with ultraviolet detection (Abstract 3318), *Pharm.Res.*, **1997**, *14*, S568.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES
Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.2
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine
Interfering: chlorpromazine

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.56 (of first (+) enantiomer)

KEY WORDS
chiral; α 1.89

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

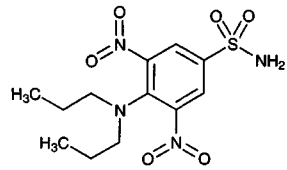
HPLC VARIABLES
Column: 150 × 3.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Detector: UV 199

CHROMATOGRAM
Retention time: k' 3.90

OTHER SUBSTANCES
Simultaneous: hyoscyamine, bromocriptine, benztropine, biperiden
Noninterfering: amantadine, carbidopa, levodopa

REFERENCE
Selinger,K.; Lebel,G.; Hill,H.M.; Discenza,C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma, *J.Chromatogr.*, **1989**, *491*, 248–252.

Oryzalin



Molecular formula: $C_{12}H_{18}N_4O_6S$
Molecular weight: 346.36
CAS Registry No.: 19044-88-3
Merck Index: 7015

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 mL MeCN and 3 mL water. Plasma. Add 500 μL plasma to the SPE cartridge, dry with vacuum. Elute with three 200 μL portions of MeCN. Inject a 10-50 μL aliquot of the eluate. Microsomal incubations. Centrifuge microsomal incubation at 12000 g. Add 900 μL portion of the supernatant to the SPE cartridge, dry with vacuum. Elute with three 200 μL portions of MeCN. Inject a 10-50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:water 53:47
Flow rate: 2
Injection volume: 10-50
Detector: UV 254; MS, Finnigan TSQ tandem mass, APCI, vaporizer 450°, corona discharge current 5 μA, CID, neutral argon -31 eV

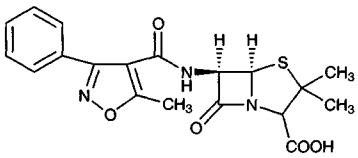
CHROMATOGRAM
Retention time: 4.7
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; mouse; rat; liver; pharmacokinetics

REFERENCE
Dvorakova,K.; Dorr,R.T.; Gallegos,A.; McClure,T.; Powis,G. Pharmacokinetic studies of the herbicide and antitumor compound oryzalin in mice, *J.Chromatogr.B*, **1997**, *696*, 275–281.

Oxacillin



Molecular formula: $C_{19}H_{19}N_3O_5S$
Molecular weight: 401.44
CAS Registry No.: 66-79-5, 7240-38-2 (Na salt monohydrate), 1173-88-2 (Na salt)
Merck Index: 7036
Lednicer No.: 1 413

SAMPLE
Matrix: blood
Sample preparation: 400 µL Serum + 400 µL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 µL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: µBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, methicillin, nafcillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefachmandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS
serum

REFERENCE
Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE
Matrix: blood, milk
Sample preparation: Milk. Adjust 5 mL milk to pH 6.3 with 100 mM HCl, deproteinize with 10 mL MeCN. Centrifuge at 1932 g for 20 min and extract the aqueous phase with two 5 mL

portions of chloroform for 20 min (Caution! Chloroform is a carcinogen!). Centrifuge at 1932 g for 20 min. Evaporate the organic phase to dryness, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot. Serum. Adjust 2.5 mL serum to pH 6.3 with 100 mM HCl, deproteinize with 10 mL MeCN. Centrifuge at 1932 g for 20 min and extract the aqueous phase with two 5 mL portions of dichloromethane for 20 min. Centrifuge at 1932 g for 20 min. Evaporate the organic phase to dryness, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot

HPLC VARIABLES
Column: 15 × 3.9 4 μm Nova Pack C18
Mobile phase: MeCN:20 mM KH_2PO_4 21:79, pH 5
Flow rate: 1.2
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Retention time: 3.8
Internal standard: oxacillin

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
serum; cow; oxacillin is IS

REFERENCE
Pérez,B.; Prats,C.; Castells,E.; Arboix,M. Determination of cloxacillin in milk and blood of dairy cows by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *698*, 155–160.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma, serum. 100 μL Plasma or serum + cloxacillin + 100 μL 500 mM pH 2.2 citric acid buffer + 20 μL 500 mM HCl + 2.5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in mobile phase, inject an aliquot. Urine. Dilute urine with water, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 ODS pellicular
Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:20 mM pH 6.6 sodium acetate 34:100
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 7
Internal standard: cloxacillin (9)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dicloxacillin, flucloxacillin

KEY WORDS
plasma; serum

REFERENCE
Thijssen,H.H.W. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 339–345.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 20 μL 4% aqueous sodium dodecyl hydrogen sulfate solution, shake for 30 min, filter (Amicon MPS-1 micropartition system, YMT membrane) while centrifuging, adjust the pH of the ultrafiltrate to 6.3-6.5 with pH 4 citrate buffer, inject a 500 μL aliquot onto column A with mobile phase A and elute to waste, after 10 min elute the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. Urine. Make up 5-100 μL urine to 500 μL with water, inject onto column A with mobile phase A and elute to waste, after 10 min elute the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 4 Nucleosil 5-C18; B 250 × 5 Nucleosil 5-C18
Mobile phase: A MeCN:33 mM NaH_2PO_4 5:95; B MeCN:33 mM NaH_2PO_4 20:80
Injection volume: 500
Detector: UV 210

CHROMATOGRAM

Internal standard: oxacillin

OTHER SUBSTANCES

Extracted: penicillin V

KEY WORDS

plasma; column-switching; ultrafiltrate; oxacillin is IS

REFERENCE

Lintz,W.; Hirsch,I.; Osterloh,G.; Schmidt-Böthelt,E.; Sous,H. Bioverfügbarkeit von Penicillin V in einer wäßrigen Zubereitungsform [Bioavailability of penicillin V in aqueous dosage forms], *Arzneimittelforschung*, **1984**, *34*, 66–71.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.76

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 mm long Co:Pell ODS
Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 10.0
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, penicillin G, penicillin V

KEY WORDS
tablets; capsules; oral suspensions; injections

REFERENCE
Briguglio,G.T.; Lau-Cam,C.A. Separation and identification of nine penicillins by reverse phase liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 228–231.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 27.0 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 2 mM phosphoric acid containing 8 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 10 mL MeOH, 10 mL water, 5 mL 2% NaCl, and 5 mL 100 mM pH 8 phosphate extraction buffer. Add 30 mL 100 mM pH 8 phosphate extraction buffer to 5 mL milk, add 1.65 mL 1 M sulfuric acid to reach pH 4.0-4.5, vortex for 30 s, centrifuge at 2400 g for 10 min, add 600 μL 5 M NaOH to the supernatant to reach pH 8, vortex, centrifuge at 2400 g for 5 min. Add the supernatant

to a reservoir attached to the SPE cartridge, pull through the SPE cartridge at 3 mL/min, remove the reservoir and elute with 1 mL MeCN:water 40:60. Add 500 μL derivatizing reagent to the eluate, vortex, heat at 65° for 10 min, cool to room temperature (protect from light), inject a 100 μL aliquot of the derivatized sample. (Prepare the 100 mM pH 8 phosphate extraction buffer as follows. Dissolve 15.6 g K_2HPO_4 dihydrate in 800 mL water, adjust pH to 8 with 10 M NaOH, make up to 1 L. Prepare the derivatizing reagent as follows. Weigh out 13.78 g 1,2,4-triazole, add 60 mL water, stir, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, dilute to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)

Mobile phase: MeCN:MeOH:buffer 37:5:58 (Prepare the 100 mM pH 6.5 phosphate buffer containing 15 mM thiosulfate and 30 mM tetrabutylammonium hydrogen sulfate as follows. Weigh 4.969 g anhydrous NaH_2PO_4, 10.139 g Na_2HPO_4 dihydrate, 3.894 g sodium thiosulfate pentahydrate, and 10.186 g tetrabutylammonium hydrogen sulfate, dissolve in 800 mL water, adjust pH to 6.5 with 5 M NaOH, dilute to 1 L with water, mix thoroughly, filter under vacuum (0.45 μm).)

Flow rate: 1

Injection volume: 100

Detector: UV 340

CHROMATOGRAM

Retention time: 10

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: cloxacillin, dicloxacillin

KEY WORDS

derivatization; SPE

REFERENCE

Verdon,E.; Couedor,P. Determination of isoxazolylpenicillins residues in milk by ion-pair reversed-phase high-performance liquid chromatography after precolumn derivatization, *J.Chromatogr.B*, **1998**, *705*, 71–78.

SAMPLE

Matrix: milk

Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 mL MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 7.21
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: penicillin G, phenethicillin, methicillin, cloxacillin, dicloxacillin, nafcillin
Interfering: penicillin V, phenethicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES
Column: 100 × 8 Radial-Pak 10 μm μBondapak C18
Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 13.4
Limit of detection: 0.2 ppm

OTHER SUBSTANCES
Extracted: penicillin V, ampicillin, methicillin, penicillin G, cloxacillin, nafcillin, dicloxacillin

KEY WORDS
column-switching

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water,

elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 33.10

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Al-Obaidy,S.S.; Po,A.L.W.; McKiernan,P.J.; Glasgow,J.F.T.; Millership,J. Assay of paracetamol and its metabolites in urine, plasma and saliva of children with chronic liver disease, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1033–1039.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 33.10

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE

Matrix: milk

Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)

Column temperature: 30

Flow rate: 1

Injection volume: 150

Detector: UV 323

CHROMATOGRAM

Retention time: 34

Internal standard: penicillin V (28.5)

Limit of detection: 1.4 ng/mL

Limit of quantitation: 1.9 ng/mL

OTHER SUBSTANCES

Extracted: amoxicillin, ampicillin, cloxacillin, dicloxacillin, penicillin G

KEY WORDS

derivatization; cow; SPE

REFERENCE

Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE

Matrix: perfusate

Sample preparation: 200 μL Perfusate + 300 μL MeCN, mix, centrifuge for 5 min, inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 3.6 LiChrosorb RP-2

Column: Chemcosorb 5-ODS-H (Chemco, Japan)
Mobile phase: MeOH:pH 5.2 acetate buffer 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 230

CHROMATOGRAM
Limit of quantitation: 2 μg/mL

KEY WORDS
rat; liver; pharmacokinetics

REFERENCE
Yasui,H.; Yamaoka,K.; Fukuyama,T.; Nakagawa,T. Effect of liver intoxication by carbon tetrachloride in hepatic local disposition of oxacillin using moment characteristics as index, *Drug Metab.Dispos.*, **1995**, *23*, 779–785.

SAMPLE
Matrix: perfusate
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Chemcosorb 5-ODS-H (Chemco, Osaka)
Mobile phase: MeOH:acetate buffer 50:50, pH 5.2
Flow rate: 1
Injection volume: 15
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 3 μg/mL

KEY WORDS
rat

REFERENCE
Ohata,Y.; Yamaoka,K.; Yasui,H.; Nakagawa,T. Consideration on moments of outflow profile in liver perfusion system with change in perfusate flow rate using oxacillin as model drug, *Biol.Pharm.Bull.*, **1996**, *19*, 83–87.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1

Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: carbenicillin, cephapirin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 25:75
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: penicillin V

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to stand for 30 min. Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 μL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES
Column: A 4 × 4 5 μm LiChrospher 100 RP-18e; B 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 7.1
Limit of detection: 2.7 ng

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, penicillin V, penicillin G

KEY WORDS
post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE
Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.45 μm), inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 5 LiChrosorb RP-2
Column: 250 × 4.6 LiChrosorb RP-18
Mobile phase: MeOH:30 mM pH 5.6 acetate buffer 33:66
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; human; pharmacokinetics

REFERENCE
Murai,Y.; Nakagawa,T.; Yamaoka,K.; Uno,T. High performance liquid chromatographic analysis and pharmacokinetic investigation of oxacillin and its metabolites in man, *Chem.Pharm.Bull.(Tokyo)*, **1981**, *29*, 3290–3297.